# EXHIBIT B

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**Applicants:** Kathryn E. Lawlor, et al. **Examiner:** Cherie M. Woodward

**Serial No:** 10/525,363 **Art Unit:** 1647

**Filed:** September 6, 2005 **Docket:** 18688

**For:** A METHOD FOR TREATMENT AND PROPHYLAXIS OF ARTHRITIS BY INHIBITING ACTIVITIES OF G-CSF AND/OR G-CSFR

**Confirmation No.:** 5197

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

## DECLARATION OF DR. IAN WICKS UNDER 37 C.F.R. §1.132

Sir:

I, Dr. Ian Wicks, hereby declare as follows:

1. I hold a medical degree (Bachelor of Medicine and Surgery, University of Sydney), specialist medical qualifications as a Rheumatologist (Fellowship, Royal Australasian College of Physicians) and a Doctorate in medical biology (PhD, University of Melbourne). I am an international fellow of the American College of Rheumatology. I am employed as a National Health and Medical Research Council of Australia Clinical Practitioner Fellow at the Walter & Eliza Hall Institute of Medical Research. I have a dual appointment as Professor/Director of Rheumatology at the Royal Melbourne Hospital (first appointed 1998). I am a professorial associate at the University of Melbourne. I have been employed by WEHI for approximately 15 years. My scientific research focuses on the immunopathogenesis and treatment of rheumatoid arthritis. I have authored 76 publications. A true and correct copy of my curriculum vitae is attached hereto.

2. I am co-inventor of the subject matter embodied and claimed in above-identified patent application (hereinafter "the '363 application). I have read and understood the specification and figures of the present application. I have reviewed

the pending claims. I am entirely familiar with the subject matter disclosed and claimed in the '363 application. The claims relate generally to a method for the treatment of arthritis in a subject by administering to the subject an agent selected from an antibody to G-CSF, an antibody to G-CSFR and a soluble G-CSFR or a G-CSF binding fragment of G-CSFR. I have also read the Office Action dated April 15, 2009. I have been asked to review and comment on the prior art cited against the '363 application in the recent Office Action.

3. It is my understanding that the Examiner holds the opinion that U.S. Patent Publication No. 20070059280 to Devalaraja, et al. (hereinafter "the '280 publication") teaches the same invention as claimed in the pending '363 application. The Examiner states further, that her opinion is evidenced by abstracts to Luross, et al. (Luross et al.; Immunology 2001 Aug; 103(4): 407-16 (2001)) as well as Meisenberg, et al. (Blood; 79(9):2267-2272 (1992)) and Campbell, et al. (J. Leukoc. Biol; 68(1):144-150(2000)).

4. As a scientist skilled in the art of rheumatology and immunology, it is my opinion that based on a reading of the scientific literature extant at the effective date of the '363 application, one skilled in the art reading the '280 publication would conclude that the '280 publication (even in view of the abstract citations identified in the pending Office Action) does not enable the practice of the presently claimed invention of the '363 application to those skilled in the art.

5. The experiments disclosed by Devalaraja et al. in the '280 publication address potential synergistic effects of a number of exogenously administered cytokines, including M-CSF, GM-CSF and G-CSF, on IL-8 induced neutrophil recruitment. Researchers in the field would view the in vitro experiments on synergy between IL-8 and G-CSF (Fig 1-4, 6-7, 9-11) skeptically because of potential in vitro artefacts. The assay on leucocyte recruitment in the skin of the ear (Fig 5) in response to administration of IL-8 plus G-CSF does not represent an accepted animal model of disease. The effects of G-CSF itself were not evaluated and the proposed therapeutic effect depends entirely on positive synergy with IL-8. Paragraph [0155], which bridges pages 10 and 11 of Devalaraja et al. ('280 publication), summarises the results (Figures 1 and 3 to 13) described in the previous paragraph [0154] as indicating that G-CSF "significantly potentiates IL-8 specific chemotaxis" of neutrophils. Paragraph [0154] in the first sentence states that the experiments were conducted to confirm that

G-CSF synergises with IL-8 induced chemotaxis in vivo and in vitro. However, on the basis of the data shown in Figures 8 and 12, Devalaraja et al also conclude that "...the data also show that pre-incubation of neutrophils with G-CSF...desensitised the neutrophils for subsequent IL-8 activation" (paragraph [0155], page 11 lines 5 to 7). This conclusion contradicts the specific claim of positive synergy between the cytokines that would promote inflammatory responses. Inhibitors of G-CSF would therefore be expected to enhance the pro-inflammatory effects of IL-8, which is the opposite of the intended effect in treating inflammatory diseases. On this basis, one might administer G-CSF to block the effect of IL-8 i.e., before any inflammation was present and G-CSF antagonists would be expected to enhance pro- inflammatory effects of IL-8. A skilled researcher in this field would not be persuaded by the data presented and the contradictory conclusions drawn. I believe that there is a huge and scientifically unacceptable leap between these data and an argument for therapeutic antagonism of endogenous G-CSF in inflammatory disease states.

6. The Examiner states that there is no difference between endogenous and exogenous G-CSF, which "has a well-defined effect of driving bone marrow granulocyte production". This has been studied for bone marrow effects of G-CSF (Meisenberg et al.; Blood 1992 May 1; 79(9): 2267-72). However, the teachings of the '280 publication are based on a synergistic effect of G-CSF on IL-8 induced recruitment of neutrophils from the peripheral circulation into the peripheral tissues. It is unlikely that endogenously derived and exogenously administered G-CSF would have identical effects in inducing recruitment of neutrophils into tissues because local tissue cytokine and chemokine gradients are required to attract neutrophils from one compartment into the other. Acute effects from high and probably non-physiological concentrations of administered G-CSF may bear little resemblance to how, when and where G-CSF is produced and regulated in vivo during a complex disease process.

7. Animal models of complex human autoimmune diseases integrate multiple, complex biological effects into a disease outcome for the animal and are therefore used to approximate condition in a human. As discussed by Luross et al. (Luross et al.; Immunology 2001 Aug; 103(4): 407-16), collagen induced arthritis (CIA) is a widely accepted animal model of human rheumatoid arthritis (RA), because CIA

relies on multiple biological responses, including those mediating inflammation and autoimmunity, that are similar to human RA.

8. Autoimmunity to type II collagen (CII, a type of collagen found in joint cartilage) is the essential driver of CIA and requires cells of the adaptive immune system, including dendritic cells and other antigen presenting cells (APCs), T cells and B cells. Campbell et al., (Campbell et al., Eur J Immunol 2000; 30:1568-1575). APCs present peptides derived from CII to autoreactive T cells via cell surface MHC molecules. In permissive situations (which remain poorly understood), T cell activation ensues, leading to help for B cells to produce pathogenic, complement fixing anti-CII antibodies. Deposition of these autoantibodies causes local complement activation within the joint environment, which attracts inflammatory cells. Autoimmunity to CII thus induces joint inflammation, which involves activation and recruitment of cells of the innate immune system, such as macrophages and neutrophils, as well as vascular endothelial cells and other stromal cell types (eg synovial fibroblasts), immunoglobulin Fc receptors and the complement cascade. For many years, macrophages and macrophage related cytokines, such as TNF and GM-CSF, have been widely thought to be the major pathological pathways by which the innate immune system contributes to the autoimmunity that causes CIA and human RA.

9. While G-CSF was initially described as a haemopoeitic growth factor, subsequent research indicated that it could have other effects that, directly or indirectly, might influence the cell types and biological pathways operating during complex, autoimmune mediated inflammatory disease states, such as CIA. In fact, the most likely prediction from the literature of the time of the invention of the '363 application was that G-CSF had immunoregulatory effects on dendritic cells and T cells that would favor immunosuppression in CIA.

10. The CSFs, including G-CSF, were discovered and characterized through effects on bone marrow precursor cells and were later introduced into clinical practice as growth factors. It was initially thought that G-CSF might activate leucocytes and thereby worsen syndromes such as septic shock. However, pre-treatment with G-CSF was found to be protective against LPS-induced organ failure, even in non-neutropaenic animals. (Gorgen et al., (J Immunol 1992; 149:918)). In follow up

studies on healthy human volunteers, G-CSF stimulation of peripheral blood cells showed reduced pro-inflammatory responses. (Hartung et al., (Blood 1995;85:2482-2489)). In clinical practice, it was observed that graft versus host disease was not a problem following G-CSF treatment, in spite of the presence of many T cells in the donor marrow. In 1995, Pan et al., (Pan et al., Blood 1995;82:4422-4429) published that G-CSF treatment polarized these T cells to an anti-inflammatory (Th2) phenotype. In 1997, it was found that blood monocyte/macrophage CD14+ cells derived from G-CSF treated donors suppressed alloantigen induced proliferation of CD4+ T cells. (Mielcarek et al., Blood 1997;89:1629-1634). In 1999, Reyes et al., (Reyes et al., British Journal of Cancer 1999;80:229-235) found that G-CSF increased human peripheral blood neutrophils, lymphocytes and monocytes, but it actually reduced mitogenic responses in the mononuclear cells. Arpinati et al., (Arpinati et al., Blood 2000; 95:2484-2490) reported induction of an anti-inflammatory phenotype in dendritic cells derived from G-CSF treated donors. In 2000, Pulendran et al., (Pulendran et al., Journal of Immunology 2000; 165:566-572) found that G-CSF preferentially promoted a subset of dendritic cells that induced production of the anti-inflammatory cytokine IL-10 by T cells. In 2002, it was found that G-CSF suppressed the release of IL-1 and TNF by LPS stimulated human whole blood cells, possibly by inhibition of proteolytic processing of IL-1 into its mature form and by translational silencing of TNF messenger RNA. (Boneberg E-M and Hartung T, Eur J Immunol 2002; 32:1717-1725). In 2003, Franzke et al., (Franzke et al., Blood 2003; 102;734-739) showed that expression of GATA-3, the transcription factor associated with a Th2 immune response, was induced in human T cells on exposure to G-CSF. These authors concluded that "G-CSF is a strong immune regulator of T cells and a promising therapeutic tool in acute graft versus host disease as well as in conditions associated with Th1/Th2 imbalance, such as...autoimmune diseases". In the introduction to their paper in 2004, Morris et al., (Morris et al., Blood 2004;103:3573-3581) refer to a number of potential mechanisms behind the consistent observation that G-CSF decreases GVHD (graft versus host disease), including Th2 polarization, effects on dendritic cells, monocytes, natural killer cells, natural killer T cells, plus the possibility of direct effects on T cells.

11. The literature of the time therefore strongly suggested that G-CSF had properties that extended beyond stimulation of bone marrow progenitor cells and

included immunoregulatory effects on a number of cells known to be crucial mediators of immunological responses, namely dendritic cells and T cells.

12. G-CSF treatment was also evaluated in the context of animal models of autoimmune disease. In lupus prone MRL-lpr/lpr mice, (Zavala et al., J Immunol 1999:163:5125-5132), chronic treatment with varying doses of G-CSF had different effects. Low dose treatment worsened several disease parameters; but conversely, high dose treatment lessened kidney damage and significantly prolonged survival of the mice. The authors concluded that high doses of G-CSF may have a beneficial effect "in the prevention of lupus nephritis, that may hold promise for future clinical applications..."

13. Adjuvant induced arthritis (AIA) is a widely studied model of RA, which can be induced with immunization of susceptible rats. Treatment of rats with G-CSF decreased disease severity of AIA, associated with reduced antigen presenting capacity (Brendolan et al., Cell Immunol 2003:221:6-14). Experimental autoimmune encephalomyelitis (EAE) is a murine model of multiple sclerosis (MS). Using gene microarrays, Lock et al., (Lock et al., Nature Medicine 2002;8(5):500-508) unexpectedly found increased expression of G-CSF in acute brain lesions from MS autopsy specimens. Pre-treatment of mice with G-CSF prevented EAE. The authors noted that reversal of ongoing EAE had been previously reported, (Zavala et al., J Immunol 2002;168:2011-2019) and concluded that "G-CSF production in acute EAE and acute MS may be one of the regulatory molecules that naturally suppress acute attacks".

14. In contrast, Campbell et al. (Campbell et al., J Leuk Biology 2000;68:144-150) reported that treating mice already immunized to develop CIA with very high (and probably non-physiological) doses of G-CSF, worsened disease, although the biological mechanisms involved were not addressed. Campbell et al. noted that results with exogenous G-CSF could not be used to infer a role for endogenous G-CSF and state specifically on page 149 that "additional studies are required to determine whether endogenous G-CSF is necessary for CIA development". This is consistent with my conclusions at Paragraph 6 herein regarding endogenous and exogenous G-CSF.

15. When considered together, in view of the teachings of the '280 publication, these studies demonstrate to any one skilled in the art that the immunological effects of G-CSF and hence the net effect on disease outcome in animal models of autoimmune mediated diseases, was not reasonably predictable at the time of the present invention. On balance, the weight of evidence favored immunoregulatory effects from G-CSF that would suppress autoimmune diseases. Consequently, those skilled in the art, until the present invention, remained quite skeptical of the potential to treat inflammatory disease and in particular, autoimmune mediated inflammatory arthritis, by blocking G-CSF signalling. The field was greatly surprised at the efficacy of inhibitors of G-CSF in the relevant in vivo disease models disclosed in the '363 application.

16. Accordingly, it is my scientific opinion that those skilled in the art would conclude that the teachings of the '280 publication do not support, and are not enabling for, the invention claimed in the pending '363 application. This opinion is entirely supported by the literature extant at the time of the filing of the '363 application.

17. I declare that all statements herein of my own knowledge are true and that all statements made on information and behalf are believed to be true; and that those statements were made with the knowledge that willful false statements are punishable by fine and imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 14/10/09

IWicks

Dr. Ian Wicks

References

1. Devalaraja et al.; US Patent Application Publication 20070059280
2. Luross et al.; (Immunology 2001 Aug; 103(4): 407-16)
3. Campbell et al., (Eur J Immunol 2000;30:1568-1575)
4. Gorgen et al., (J Immunol 1992; 149:918)
5. Hartung et al., (Blood 1995;85;2482-2489)
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